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Surface Structure of Ascospores of Genus *Nadsonia* Sydow

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The surface structure of ascospores of *Nadsonia commutata* IFO 10029[T] (JCM 10138) and IFO 10030 (JCM 10139), *N. fulvescens* var. *elongata* IFO 10894 (JCM 9991), and *N. fulvescens* var. *fulvescens* IFO 10895 (JCM 10023) was viewed under a scanning electron microscope. Ascospores of *N. commutata* were covered with irregular protuberances, while the two varieties of *N. fulvescens* were ornamented with stellate spines.

Key words : *Nadsonia commutata, Nadsonia fulvescens* var. *fulvescens, Nadsonia fulvescens* var. *elongata*, surface structure of ascospores

INTRODUCTION

In 1911, Nadson and Konokotina (14) isolated a strain of yeast from exudate of an oak in the neighborhood of St. Petersburg and named it *Guilliermondia fulvescens*. In 1912, Sydow (15) changed the generic name *Guilliermondia*, which was a later homonym, to *Nadsonia*. In 1913, Konokotina (7) isolated a yeast from exudate of a birch in the province of Smolensk and named it *Nadsonia* (*Guillimondia*) *elongata*. In 1973, Golubev (4) isolated several strains of this genus from field soil of East Falkland and classified them into a new species *Nadsonia commutata*.

In 1989, Golubev et al. (5) carried out a detailed taxonomic study of the genus *Nadsonia* on the basis of morphology, physiology, cellular amino acid and fatty acid composition, electrophoretic patterns of some enzymes, and DNA relatedness, and found two species had two varieties, i. e., *N. commutata, N. fulvescens* var. *fulvescens*, and *N. fulvescens* var. *elongata*.

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The genus *Nadsonia* is characterized by the unique process of sporulation and morphology of ascospores, and by lemon-shaped cells proliferated by bipolar budding from the broad base. Taxonomic significance of surface structure of ascospores by using scanning electron microscopy have been reported by several authors, for example, on *Schwanniomyces* (8), *Saccharomyces uvarum* (2), *Debaryomyces* (9), *Torulaspora*-group of *Saccharomyces* (9), *Debaryomyces hansenii* (1), *Pichia membranaefaciens* (10), *Schizosaccharomyces* (11), and *Metschnikowia* (12).

In their detailed taxonomic studies on the genus *Nadsonia*, however, Golubev et al. (5) did not examine the surface structures of ascospores. They stated that neither ascospore formation nor conjugation was observed in the strains of *N. fulvescens* var. *fulvescens*. According to Golubev (personal communication), strains of *N. fulvescens* var. *fulvescens* which are now maintained in culture collections are assumed to have originated from a single strain isolated by Nadson and Konokotina (14), and do not sporulate anymore. Recently, a sporulating strain of this variety was isolated from a moss collected in Japan. This enabled us to study the

Table 1. Key characteristics of the varieties of *Nadsonia fulvescens*

	N. fulvescens var. *elongata*		*N. fulvescens* var. *fulvescens*	
	Reference*	IFO 10894	Reference	IFO 10895
Fermentation				
Glucose	+	+	+	+
Galactose	−	−	+	+
Maltose	−	−	+	+
Assimilation				
Glucose	+	+	+	+
Galactose	−	−	+/s	+
L-Sorbose	+/s	s	+	+
Sucrose	−	−	w	+
Maltose	−	−	+	+
Mannitol	−	−	+	+
Glucitol	w/−	+	+	s
α-Methyl-D-glucoside	−	−	+/s	+

* Golubev et al. 1989 (5), + : positive, s : slow, w : weak, − : negative

surface structure of the known species and varieties of *Nadsonia*.

MATERIALS AND METHODS

Strains examined. Four strains of *Nadsonia* were examined. *Nadsonia commutata* IFO 10029^T (JCM 10138) and IFO 10030 (JCM 10139) were received from W. I. Golubev. *Nadsonia fulvescens* var. *elongata* IFO 10894 (← JCM 9991) was received from C. P. Kurtzman as *N. fulvescens* var. *fulvescens* NRRL Y-991. This strain was later identified as *N. fulvescens* var. *elongata* by Golubev et al. (5). *Nadsonia fulvescens* var. *fulvescens* IFO 10895 (← JCM 10023) was isolated by T. Nakase from a moss *Pyrrhobryum dozyanum* (Lac.) Manuel collected in the forest of a temple, Kasamori-kannon, Chiba Pref., Japan, in Mar., 1995.

Sporulation. A mass of cells harvested from a colony on a YM agar slant was transferred to another YM agar slant and incubated for 1 to 2 weeks at 15°C. The colony turned to brown due to abundant sporulation.

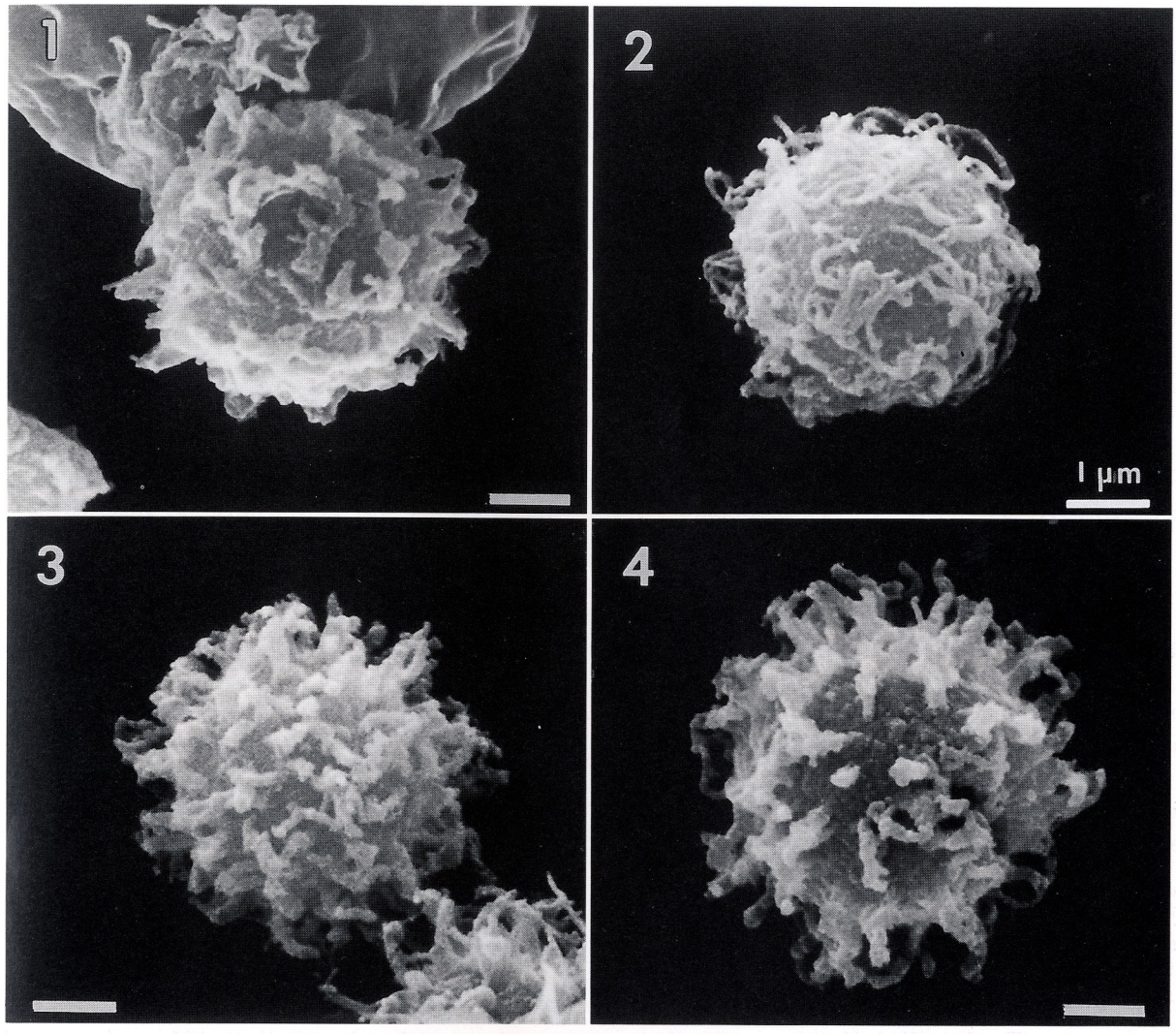
Preparation of ascospores for SEM. After confirmation of sufficient production of ascospores using a light microscope, the ascogenous cells were treated with an enzyme mixture of 1 mg of Zymolyase-100 T (Seikagaku Co.), 10 mg of Novozyme 234 (Novo Nordisk) and 10 mg crude enzyme of *Trametes sanguinea* (3) per m*l* for 2 h at 37°C to digest ascus wall, and washed twice with 0.1 M phosphate buffer (pH 7.2) by centrifugation. The free ascospores were then fixed with 2% glutaraldehyde in the phosphate buffer for 2 h at 4°C and 1% osmic acid in the phosphate buffer. The fixed samples were dehydrated by passing through a graded acetone concentration series of 30, 50, 60, 70, 80, 90, 95, and 100% at 15 min intervals, and then were transferred to isoamylacetate for more than 2 h. The dehydrated samples of ascospores were mounted on a small disk of cover glass and subjected to critical point drying in an HCP-2 (Hitachi Ltd.). The glass disk with the dried ascospores was coated with platinum at 10 mA for 3.5 min in vacuo (4 Pa.) using ion sputtering apparatus JUS-5000 (JEOL Ltd.).

The preparations were examined under a scanning electron microscope JSM-5400 (JEOL Ltd.) at a voltage of 15 KV.

RESULTS AND DISCUSSION

To confirm the taxonomic status of *N. fulvescens* var. *fulvescens* IFO 10895, the physiological properties were examined by the standard method described by van der Walt and Yarrow (16). Key characteristics of this strain are shown in Table 1, which exhibits physiological differences between the two varieties.

Nadsonia commutata IFO 10029^T (JCM 10138) and

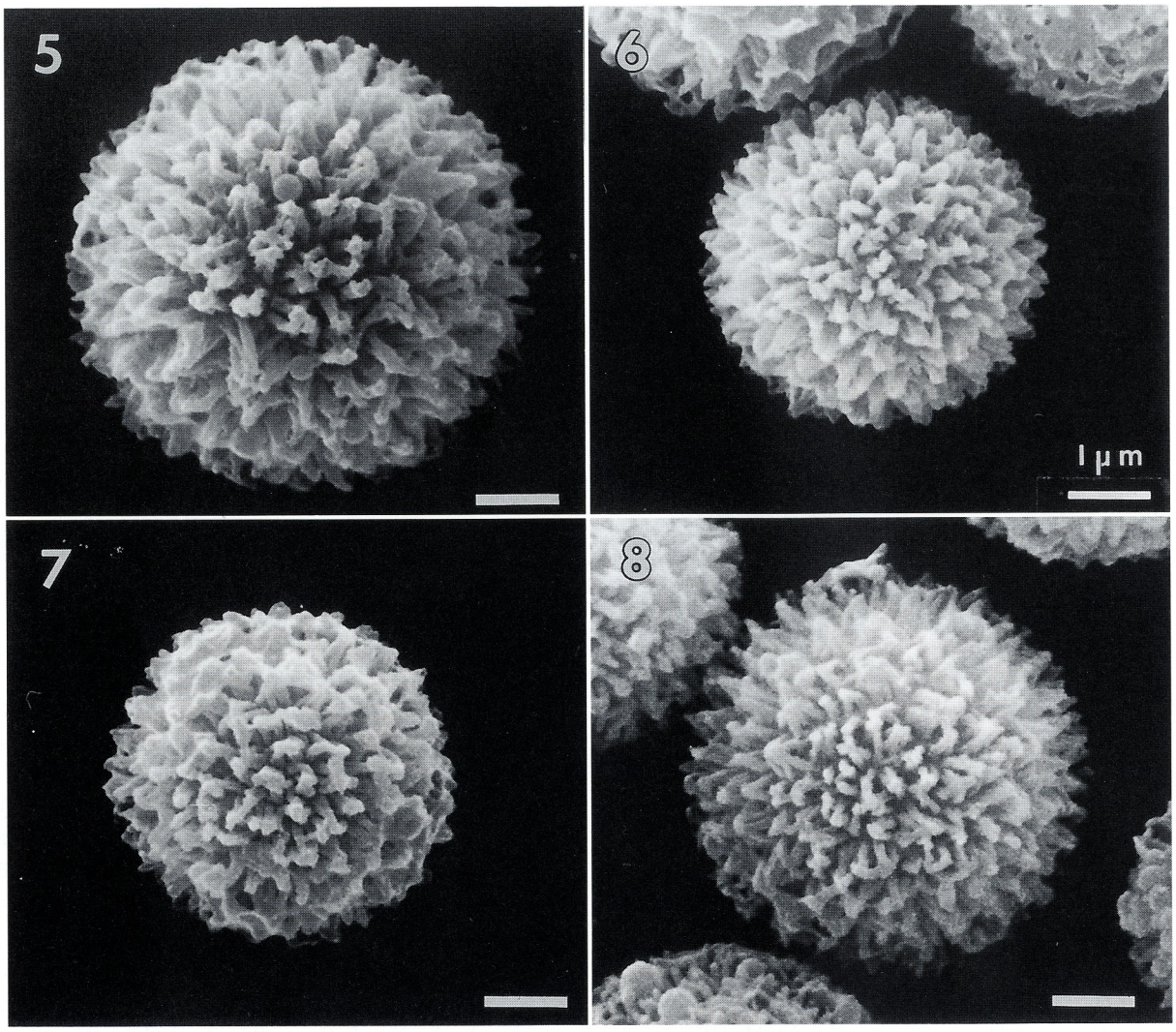


Figs. 1-4. Scanning electron micrographs of ascospores of *Nadsonia commutata*
1 and 2, *N. commutata* IFO 10029^T (JCM 10138), 3 and 4, *N. commutata* IFO 10030 (JCM 10139). Scale bars : 1 μm

IFO 10030 (JCM 10139) produced ascospores after heterogamic conjugation between the mother cell and a bud, which is normally delimited by a septum. The mother cell becomes the ascus. One, or rarely two, spherical, brownish, spiny- to warty-walled ascospores are produced. The ascospores contain a prominent lipid globule. These observations coincide well with the observation by Miller and Phaff (13).

Two strains of *N. commutata* were resistant to the lytic enzyme mixture used. Therefore, ascus-wall was disrupted by gentle shaking with glass beads after enzyme treatment. The surface of ascospores is covered with filamentous or irregular protuberances (Figs. 1-4). These structures slightly resemble the hair-like pattern found on ascospores of *Debaryomyces hansenii* (1), but are distinguishable.

Since ascospores could not be found in *N. fulvescens* var. *elongata* in IFO, IFO 0665^T and *N. fulvescens* var. *fulvescens* IFO 0666^T in our collection, we requested JCM to provide sporulating strains of these varieties. *Nadsonia fulvescens* var. *elongata* IFO 10894 (JCM 9991) and *N. fulvescens* var. *fulvescens* IFO 10895 (JCM 10023) produce asci and ascospores after heterogamic conjugation between



Figs. 5-8. Scanning electron micrographs of ascospores of *Nadsonia fulvescens*
5 and 6, *N. fulvescens* var. *elongata* IFO 10894 (JCM 9991), 7 and 8, *N. fulvescens* var. *fulvescens* IFO 10895 (JCM 10023). Scale bars : 1 μm

the mother cell and a bud. After incubation for 1 to 2 weeks at 15°C, the rate of asci was 50 to 80% of the cells. The contents of the zygote move to another bud formed at the opposite end of the mother cell. This second bud is delimited by a septum and becomes an ascus. One, rarely two, spherical, brownish, spiny- to warty-walled ascospores are produced. The ascospore contains a prominent lipid globule. These observations coincide well with those reported by Miller and Phaff (13).

After treatment with Zymolyase, asci of these two strains retain the thin film around the ascus. Further treatment of Novozyme and crude enzyme of *Trametes sanguinea* exposed the naked ascospores. The surface of ascospores is ornamented with stellate spines (Figs. 5-8). This surface structure has not been reported in yeasts. Our observations revealed that the two varieties of *N. fulvescens* have the same surface structure as ascospores, which may support the classification of Golubev et al. (5) in which *N. fulvescens* and *N. elongata* are included in the same species as respective varieties.

Kawakami (6) studied the fine structure of ascospores of *N. fulvescens* NRRL Y-991 under a transmission electron microscope and reported that

it was a gear type having a hair-like process (protuberance). The electron micrographs showed cross-sections of the surface protuberance of ascospores, namely, pictures of triangle, isosceles triangle and triangle with concaved apex, which correspond to the structure we observed by SEM on the surface of ascospores. They pointed out that this kind of surface structure had not been reported yet.

The surface structure of *N. fulvescens* resembles the following structures in some respects : wart-like protuberances found in ascospores of *Debaryomyces hansenii* (1, 9), *Torulaspora globosa* (9), and *Schizosaccharomycs pombe* (11) ; and ridges and blunt protuberances found in ascospores of *D. cantarellii* (9), *D. castellii* (9, 11), *D. coudertii* (9), *D. polymorphus* (9), *D. vanrijiae* (9), *T. delbrueckii* (9) ; and wart-like protuberances found in *S. pombe* (11). However, the surface structure of *N. fulvescens* is unique and apparently different from those of yeasts mentioned above.

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Nadsonia 属酵母の子嚢胞子の表面構造

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Nadsonia commutata IFO 10029^{T} (JCM 10138), IFO 10030 (JCM 10139) の 2 株と *N. fulvescens* var. *elongata* IFO 10894 (JCM 9991) および *N. fulvescens* var. *fulvescens* IFO 10895 (JCM 10023) について，走査型電子顕微鏡を用いて子嚢胞子の表面構造を調べた．*N. commutata* の子嚢胞子の表面は全体に不規則な突起物によって覆われていた．*N. fulvescens* の 2 変種の子嚢胞子の表面は全体に星型の規則正しい突起物によって覆われていた．これらの表面構造は酵母の中で新規な形態であった．